REMARKS

In the Office Action dated January 20, 2004, claims 1-26, in the above-identified U.S. patent application were rejected. Reconsideration of the rejections is respectfully requested in view of the above amendments and the following remarks. Claims 1-44 have been canceled and new claims 45-89 have been added to the application.

Figures 1a and 1b were objected to. New copies of these figures are attached. The new figures are the same as the originally filed figures but have been lightened to reduce the background.

Claims 1-26 were rejected under 35 USC §112, second paragraph as indefinite. Claims 1-26 have been canceled and new claims added to the application which clarify most of the language which was found indefinite. However, applicants point out that the language "amplification products" is generally known in the art to mean nucleic acids which are produced as a result of an amplification reaction such as PCR. Thus, applicants contend that this term is not indefinite. Claim 21 was rejected due to the term "nucleic acid". The office action contends that this term means a single nucleic acid as compared to a nucleic acid strand. Attached to this response is a reference, Ullmann's Encyclopedia of Industrial Chemistry, which indicates that nucleic acids are long unbranched chains of sugar and phosphate. Applicants contend that the term "nucleic acid" is often used in the art to indicate a strand of nucleic acids not a single purine or pyrimidine. However, in order to clarify this, the new claims use the language "nucleic acid strand". Regarding the rejection of the language "in

the presence of a salt and polyethylene glycol", applicants point out that such language is commonly used in the art to mean that the salt and polyethylene glycol are in the solution at the time that binding to the solid phase occurs. In other words, they could be added to the nucleic acid solution or to a solution on the solid phase, and they could be added to the solid phase before the nucleic acids or after. When and how the salt and polyethylene glycol are added is not critical as long as they are present during the binding of the nucleic acids to the solid phase. The claims have been amended to indicate that the salt and polyethylene glycol are present in the solution during the binding of the nucleic acids to the solid phase. In view of the new claims and the above discussion, applicants request that these rejections be withdrawn.

Claims 1, 2, 4-13, 15-19, 21, 22, 25 and 26 were rejected under 35 USC §102(b) as anticipated by Hawkins. The present invention is a method for binding nucleic acids to a solid phase having both hydrophobic and hydrophilic groups on its surface. This method enables reversible and sequence unspecific binding of nucleic acids to the solid phase. Binding of the nucleic acids takes place via the hydrophobic groups. Hawkins indicates that the polynucleotides bind to his surface via functional groups (carboxyl or thiol groups) which are hydrophilic groups. The hydrophilic regions on the surface of the solid phase according to the present invention serve to avoid agglomeration of the solid phase and the solid phase particles, respectively, in aqueous solutions. Tests with solid phase particles which had hydroxyl groups as a coating on their surface but no hydrophobic groups, showed no binding of nucleic acids.

Applicants point out example 3 on page 17 of the present application which compares purification using the presently claimed method, with COOH-coated particles according to Hawkins. The yield is considerably higher when using the presently claimed method.

Hawkins discloses a method of binding DNA to magnetic microparticles, the surface of which is coated with carboxyl groups (claim 1). Hawkins produces his particles by first coating a magnetic metal oxide core with a silane coat. Then another functional group (preferably a carboxylic acid group) is covalently bound to all of the silane groups, leading to microparticles which have their entire surface coated with the functional groups (col 3, lines 28-31). Hawkins silane coat does not act as a hydrophobic group and does not produce the same results as the present invention as shown in example 3 of the present application. Consequently, the particles disclosed by Hawkins do **not** have hydrophobic and hydrophilic groups on their surface but only one kind of functional group, preferably carboxylic acid groups (col. 3, lines 35-39) to which the DNA is bound. In view of the fact that Hawkins does not suggest or disclose a solid phase which contains both hydrophobic and hydrophilic groups on its surface, applicants contend that Hawkins does not anticipate the presently claimed method and request that this rejection be withdrawn.

Claims 1-24 were rejected under 35 USC §103(a) as unpatentable over Hawkins in view of Tang. As discussed above, Hawkins does not suggest or disclose a solid phase which contains both hydrophobic and hydrophilic groups on its surface. Tang does not cure this deficiency as Tang was cited for the

disclosure of a method for synthesizing and purifying oligonucleotides using microparticles which have hydroxyl or amino groups. Tang is not directed to methods for binding nucleic acids to a solid phase, only to methods for synthesizing oligonucleotides. In view of the fact that neither Hawkins or Tang suggests or discloses a method for binding nucleic acids to a solid phase which contains both hydrophobic and hydrophilic groups on its surface, applicants request that this rejection be withdrawn.

Applicants respectfully submit that all of claims 45-89 are now in condition for allowance. If it is believed that the application is not in condition for allowance, it is respectfully requested that the undersigned attorney be contacted at the telephone number below.

In the event this paper is not considered to be timely filed, Applicants respectfully petition for an appropriate extension of time. Any fee for such an extension together with any additional fees that may be due with respect to this paper, may be charged to Counsel's Deposit Account No. 02-2135

Please charge any fee deficiency or credit any overpayment to Deposit Account No. 02-2135.

Respectfully submitted,

By 

Monica Chin Kitts
Attorney for Applicants
Registration No. 36,105
ROTHWELL, FIGG, ERNST & MANBECK, p.c.
Suite 800, 1425 K Street, N.W.
Washington, D.C. 20005
Telephone: (202)783-6040

Ullmann's Encyclopedia of Industrial Chemistry

Volumes 1 - 39: Alphabetically Arranged Articles
Volume 40: Index

Ullmann's Encyclopedia of Industrial Chemistry

Sixth, Completely Revised Edition

Volume 24

Nucleic Acids
to
Paints and Coatings

WILEY-VCH

Library of Congress Card No.: Applied for.

British Library Cataloguing-in-Publication Data:
A catalogue record for this book is available from the British Library.

Bibliographic information published by Die Deutsche Bibliothek.
Die Deutsche Bibliothek lists this publication in the Deutsche Nationalbibliografie; detailed bibliographic data is available in the Internet at <http://dnb.ddb.de>.

ISBN 3-527-30385-5

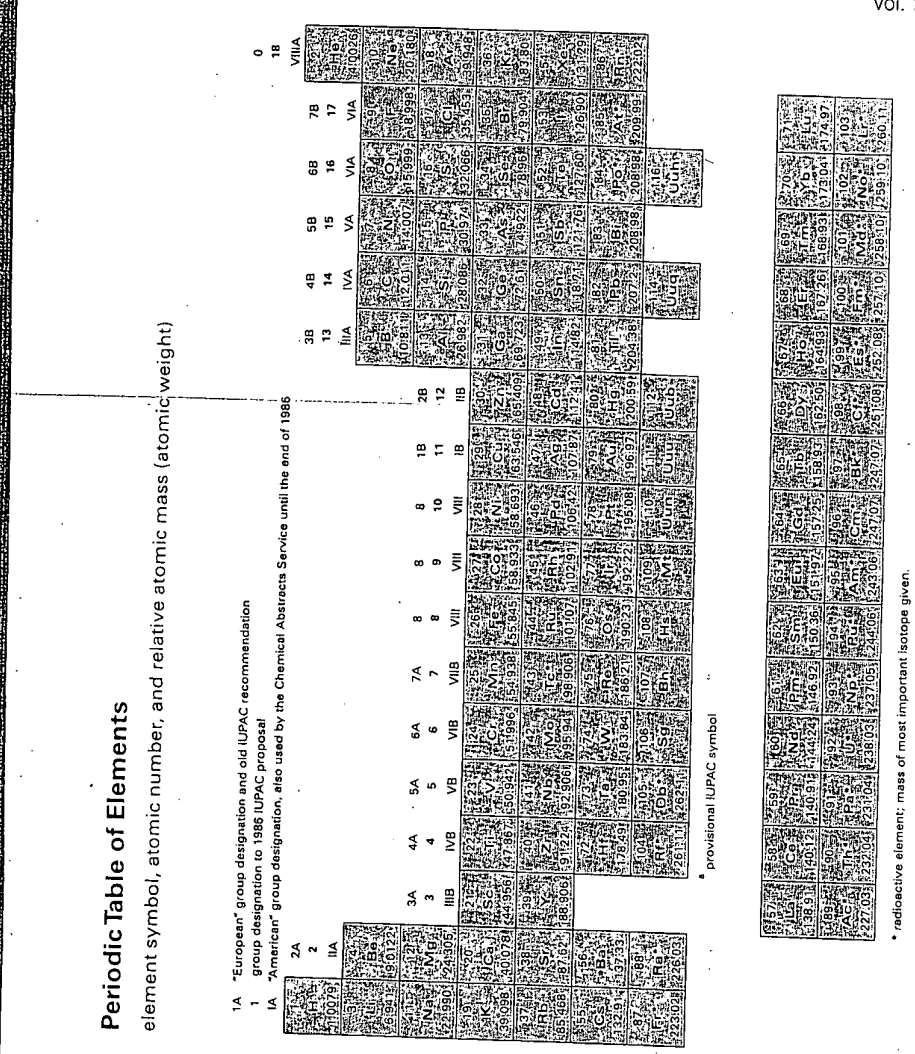
Cover Design: Gunther Schulz, Fussgönheim
Composition: Steingraeber Satztechnik GmbH, Dossenheim
Printing: Strauss Offsetdruck GmbH, Mörlenbach
Bookbinding: Litges & Dopf Buchbinderei GmbH, Heppenheim

Printed in the Federal Republic of Germany

Contents

Cross References

Nylon → Fibers, 4. Synthetic Organic **13**; → Polyamides **28**
Octanol → Alcohols, Aliphatic **2**; → 2-Ethylhexanol **12**
Odorants → Flavors and Fragrances **14**
Oil Sand → Tar Sands **35**
Oils, Essential → Flavors and Fragrances **14**
Oils, Mineral → Natural Gas **22**; → Oil Refining **24**
Olefin Polymers → Polyolefins **28**
Olefins → Butadiene **5**; → Butenes **6**; → Ethylene **12**; → Hydrocarbons **17**; → Isoprene **18**; → Propene **30**; → Styrene **34**; → Terpenes **35**
Oleic Acid → Fatty Acids **13**
Oleoresins → Resins, Natural **31**
Olivine → Silicates **32**
Oral Antidiabetics → Antidiabetic Drugs **3**
Orthoformates → Esters, Organic **12**
Orthophosphoric Acid → Phosphoric Acid and Phosphates **26**
Osmium → Platinum Group Metals and Compounds **27**
Osmosis → Membranes and Membrane Separation Processes **21**
Oxazine → Azine Dyes **4**
Oxide Ceramics → Ceramics, Advanced Structural Products **7**; → Superconductors **35**
Oxirane → Ethylene Oxide **12**
Packaging Materials → Films **13**; → Foods, 4. Food Packaging **14**; → Paper and Pulp **25**
Paint and Varnish Removers → Paints and Coatings **24**
Palladium → Platinum Group Metals and Compounds **27**
Palm Oil → Fats and Fatty Oils **13**
Palmitic Acid → Fatty Acids **13**
Pantothenic Acid → Vitamins **38**



Nucleic Acids

Genetic Engineering is a separate keyword.

HELMUT BURTSCHER, Boehringer Mannheim GmbH, Penzberg, Federal Republic of Germany (Chaps. 1 – 6)

SIBYLLE BERNER, Boehringer Mannheim GmbH, Tutzing, Federal Republic of Germany (Chap. 7)

RUDOLF SEIBL, Boehringer Mannheim GmbH, Penzberg, Federal Republic of Germany (Chap. 8)

KLAUS MÜHLEGGER, Boehringer Mannheim GmbH, Tutzing, Federal Republic of Germany (Chap. 9)

1. Introduction

Nucleic acids are high molecular mass compounds found in all living cells and viruses. Their name originates from their discovery in the nuclei of eucaryotic cells. They can be chemically degraded to yield phosphoric acid, pentoses, and nitrogen-containing heterocycles (bases). Nucleic acids can be divided into two main classes depending on the sugar they contain: *deoxyribonucleic acids* (DNA) contain 2-deoxy-D-ribose and *ribonucleic acids* (RNA) contain D-ribose.

Nucleic acids are long, unbranched chains of sugar and phosphate (Fig. 1, see next page): the C-3′ atom of each sugar is linked by a phosphodiester bond to the C-5′ atom of the neighboring sugar. Either a purine (adenine, guanine) or a pyrimidine (cytosine and thymine in DNA; cytosine and uracil in RNA) is attached to C-1′ of the sugar by a β-glycosidic bond. For a detailed description of purines and pyrimidines, see → Purine Derivatives, → Pyrimidine and Pyrimidine Derivatives.

Although nucleic acids have been known since the second half of the nineteenth century it was only in the 1940s that their importance as the carrier of genetic information became clear. Genetic engineering and improved physical and biochemical methods of analysis have led to enormous progress in the understanding of the structure of DNA, DNA – protein interactions, and gene organization, expression, regulation, and transfer. The importance of nucleic acids became even more obvious after the discovery that they can have other functions in addition to their ability to store and transfer genetic information. It is widely assumed that in the course

of evolution first RNA and then DNA came into being [36,37].

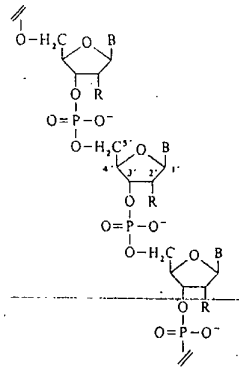


Figure 1. Structure of DNA (R = H) and RNA (R = OH)
B = base (adenine, guanine, thymine or uracil, cytosine)

2. Structure

2.1. Structure of DNA

The joining of the DNA building blocks by 5′- and 3′-phosphodiester bonds gives the molecule polarity (Fig. 1); base sequences are always written starting with the 5′-terminus, i.e., in the $5' \rightarrow 3'$ direction. The specific base sequence of DNA and its ability to form double-stranded structures according to precisely defined rules are of utmost importance for the storage of genetic information and for interactions with other nucleic acids and proteins.

From X-ray analysis data, CRICK and WATSON proposed a double-stranded structure for DNA in 1953 in which two antiparallel (i.e., $5' \rightarrow 3'$ and $3' \rightarrow 5'$) polynucleotide chains form a right-handed helix (i.e., looking along the axis of the helix, the strands are coiled clockwise). Naturally occurring DNA usually consists of right-handed helices with a major and a minor groove (Fig. 2). The hydrophobic bases are located inside the helix and the sugar–phosphate "backbone" on the outside [38]. Bases that are opposite each other are paired according to defined rules as a result of hydrogen bond formation: adenine always pairs with thymine or uracil and guanine with cytosine. Complementary bases can be bound by the more common Watson–Crick pairing (Fig. 3 A) or by Hoogsteen base pairing (Fig. 3 B). The double-stranded structure is further stabilized by hydration of the phosphate groups and hydrophobic interactions between the aromatic ring systems that result in stacking of the bases.

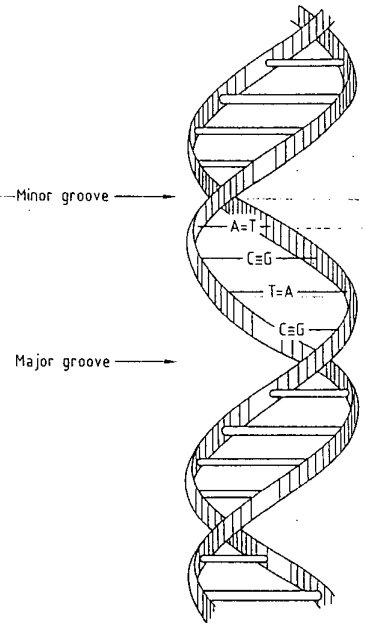


Figure 2. Right-handed double-helix of DNA
A = adenine, C = cytosine, G = guanine, T = thymine

In double-stranded DNA, the bases are densely stacked and there is a cooperative effect between hydrogen bonding and stacking. Internal bases can be continuously paired and unpaired; double-stranded regions open and form single-stranded "bubbles" ("breathing" of DNA). Breathing is more frequent in regions rich in A–T pairs and could be important for interactions with proteins. DNA helices can exist in various forms (A, B, C, D, and Z) [39] some of which are interconvertible depending on the concentration and type of salts present. The helices always exhibit a degree of microheterogeneity that plays an important part in genetic regulation mechanisms.

The DNA helices can exist in linear form (e.g., in the chromosomes of higher organisms)

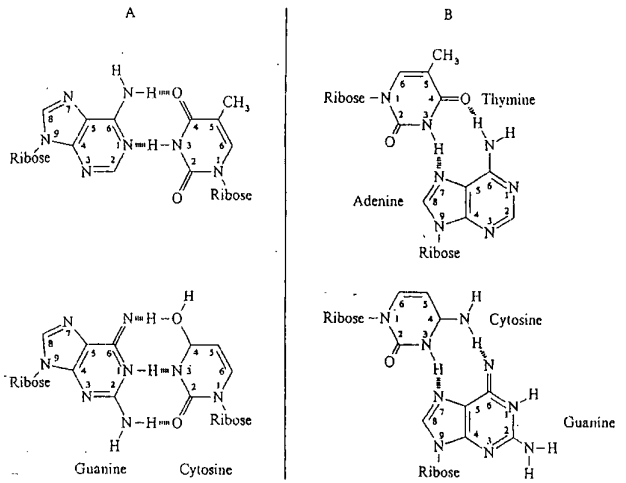


Figure 3. Watson–Crick base-pairs (A), and Hoogsteen base-pairs (B)

or as closed rings (e.g., in *Escherichia coli*); the molecules can also be twisted (superhelicity or supercoiling). In order to accommodate the large amount of DNA present in living cells, it must be packaged as compactly as possible with the help of proteins and RNA. Proteins can recognize specific binding sites on the DNA. The grooves of the DNA helix are large enough to allow proteins to come into contact with the bases [40]. Defined regions in DNA can also be recognized with the help of the methylation pattern of the bases (see Section 4.3).

Forms of DNA.

A-DNA can be observed in X-ray analyses at 66 % relative humidity. It has 11 base pairs per turn of the helix, the planes of the base pairs are tilted away from the vertical helical axis (19°), the helix is right-handed and has a diameter of ca. 2.3 nm.

B-DNA is the classical Watson–Crick form. It represents the structure of DNA at a relative humidity of > 92 % and largely corresponds to that found under physiological conditions. The helix is also right-handed with about 10.2–10.4 base pairs per turn and a diameter of ca. 2 nm. Single unpaired bases can be "looped out" of the helix and barely disturb the rest of the structure [41,42]. Protein–DNA interactions usually require recognition of nucleotide sequences in the major groove of the B-DNA double helix.

C-DNA helices can be observed at a relative humidity of 44–66 % in the presence of lithium salts. The helix is also right-handed and similar to the B form, but with 9.3 base pairs per turn.

D-DNA occurs in nature only in sequences with alternating adenine and thymine residues and in the DNA of the bacteriophage T2 (T-DNA). The helix is also right-handed and has 8 base pairs per turn.

The left-handed conformation of *Z-DNA* has an alternating sequence of pyrimidines and purines and is formed in vitro at high salt concentrations (> 2 mol/L NaCl) or in the presence of divalent cations ($Mg^{2+} > 0.7$ mol/L). Unlike the right-handed helices (which have two grooves), this structure forms a single, very deep groove that penetrates the helix axis. The sugar–phosphate backbone assumes a zig-zag arrangement (therefore Z-DNA) with 12 base pairs per turn of the helix. Z-helices can form in vivo at physiological salt concentrations. They are less stable than B-DNA, but are stabilized by supercoiling, proteins, special ions, and methylation [43]. Torsional stress of DNA in vivo can favor the formation of Z-DNA [44]. Z-DNA and B-DNA are interconvertible; part of a DNA molecule may exist in the B form and another part in the Z form.

Supercoiling. Circular DNA and DNA between fixed sites can be twisted to supercoils. The term *supercoiling* refers to the curvature of the double helix axis. Supercoiled (superhelical) DNA was discovered in the 1960s in polyoma virus [45]. Rotation in the direction of winding is called *positive supercoiling* and rotation in the opposite direction is called *negative supercoiling*.

Torsional stress due to negative supercoiling can be overcome by the formation of DNA structures other than the B form. Negative supercoiling is a strong driving force for the stabilization of Z-DNA. Supercoiling makes DNA more compact, which is very important in DNA packaging. Almost all naturally occurring superhelical DNAs are underwound (i.e., have negative superhelices) but overwound DNAs also exist [46]. The strain produced by over- or underwinding can be accommodated by the formation of local single-stranded regions which tends to increase with increasing temperature. "Breathing" of the DNA (see third paragraph in Section 2.1) plays an important part here too. A sequence with $>90\%$ A–T can exist permanently unpaired in a superhelical molecule. This is important for many reactions of DNA. Supercoiling influences transcription (see Section) and vice versa. Positive supercoils are formed in front of the transcription apparatus and negative supercoils behind it; these supercoils are controlled by enzymes [47].

Bending. The base sequence of DNA is of tremendous importance for its structure [44]. In a right-handed helix, the twist angle between two bases changes depending on the sequence. This may result in the bending of a linear double helix. Bending can also be caused by proteins. Bending is of significance for the packaging of DNA and for many of its biological reactions [48, 49].

Intrinsically bent DNA is formed when special base sequences or structural motives are repeated in phase with the DNA helical repeat; homopolymeric A tracts being the best example [50, 51]. Protein-induced DNA bending plays an important role in recombination, initiation of transcription, and replication [52, 53]. Bends are also important structural features; indeed, regulatory protein binding sites can be replaced by an intrinsic bend [49, 54].

Special Structural Elements. Short sequences are frequently repeated in regulatory regions. *Repeats* can be recognized by DNA-binding proteins. Owing to DNA breathing, double-stranded regions (hairpins or stem-loops and cruciform structures) can be formed at repeats within a single strand (Fig. 4). This rarely happens in double-stranded DNA because stem loops are energetically less favorable than linear double strands. However, it is encountered frequently in single-stranded DNA and RNA. Supercoiling can promote the formation of cruciform structures, whereas transcription inhibits it [55]. Hairpins can play a part in replication, transcription, and RNA processing [56].

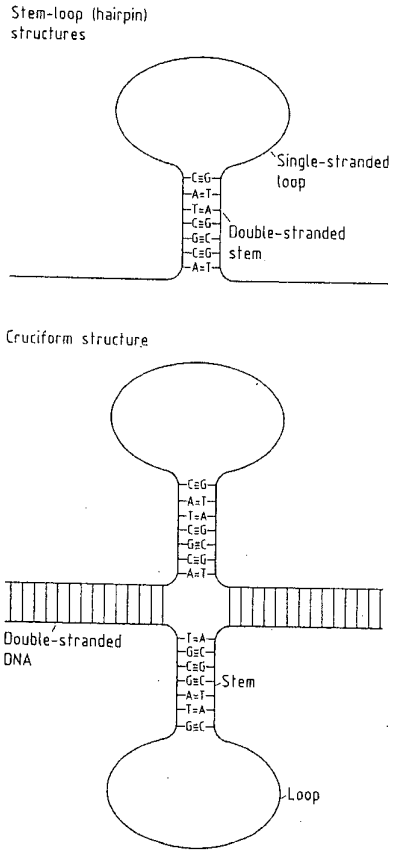


Figure 4. Special structural elements in nucleic acids

Homopyrimidine–homopurine runs are frequently found in regulatory regions of eucaryotic genes and are especially sensitive to nucleases [57]. There is a high tendency to form right-handed structures other than B-DNA in such regions [58].

Although the chains of double-stranded DNA are normally antiparallel, *parallel double-stranded oligomers* have also been found in vitro [59]. They form a right-handed helix and are even recognized by several enzymes. They are less temperature-stable than the corresponding oligomers.

At homopurine–homopyrimidine sections of the DNA, a homopyrimidine oligonucleotide can attach itself parallel to the homopurine strand in the major groove and form a *triple helix* [58]. Structures of this type can be used for specific strand cleavage with the help of coupled ellipticine derivatives or metal chelates [60, 61].

Centromeres are important compact DNA structures of the eucaryotic chromosome that are rich in adenine and thymine. Their exact structure is not known but they are important for the attachment of the spindle fibers during mitosis.

The ends (*telomeres*) of linear chromosomes (as in eucaryotic DNA) pose a special problem. DNA polymerases synthesize DNA from a DNA template and always require an RNA primer to start replication. Cleavage of this primer then results in a small 5′-gap which cannot be closed by the polymerase. Under normal replication conditions, the ends should therefore beocme shorter with every cycle of DNA replication (see Section 4.1). Special enzymes (telomerases) are responsible for adding telomere repeats to the chromosome ends to maintain constant length (Fig. 5): repeats can fold back and provide a 3′-OH group which serves as a primer for copying the last segment of a linear DNA molecule. Disturbances in telomeres can lead to aging phenomena [62, 63] and a role in carcinogenesis is also being discussed. Broken ends of chromosomes that are no longer protected by telomeres are very susceptible to fusion with other DNA ends and to degradation by nucleases [64]. The antiparallel structure and function of telomeres are highly conserved in all eucaryotes and are species specific. They consist of simple, tandemly repeated sequences with clusters of G residues [65, 66]. The G-rich strand is aligned in the $5' \rightarrow 3'$ direction towards the end of the chromosome and has a single-stranded 3′-end containing 12–16 nucleotides. Telomeres can associate to form stable, parallel, four-stranded structures (G 4-DNA) [67].

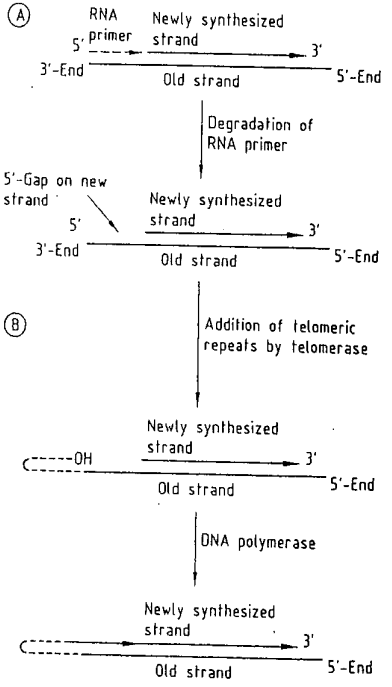


Figure 5. The importance of telomeres
Without telomere addition (A) newly synthesized DNA strands become shorter; with telomere addition by telomerase (B) constant length can be maintained.

2.2. Structure of RNA

RNA is an unbranched single-stranded polymer with many intramolecular double-stranded sections that may account for 50–67% of the molecule. As in DNA, the backbone of RNA consists of 3′,5′-phosphodiester bonds (Fig. 1); however the sugar is ribose (and not deoxyribose) and uracil replaces thymine. Double-stranded RNA cannot form a B-helix because of steric hindrance caused by the 2′-OH groups of ribose; helices of the A type are, however, possible.

The functional groups of the nucleotides in the major groove of the A type of double he-

lix found in RNA are not easily accessible to proteins [68]. Protein binding to RNA probably occurs via interaction with single-stranded regions.

Four functional RNA families exist: messenger RNA (mRNA), ribosomal RNA (rRNA), transfer RNA (tRNA), and small nuclear RNA (only in eucaryotes). The structure of tRNAs has been studied most extensively; about half of the ca. 75–90 nucleotides within the tRNA molecule are paired, resulting in a secondary structure with a stem and three loops similar to that of a cloverleaf [69].

RNA has many different biological functions and exhibits a spectrum of flexible structures that more closely resemble those of proteins rather than those of the chemically related DNA [70]. RNAs have secondary structures–double-stranded sections, hairpins, internal loops, and bulged bases. With unpaired nucleotides pronounced tertiary structures are formed in addition to the secondary structure. Examples of tertiary structure motives are pseudo knots [71]; produced by folding back in a hairpin and formation of a second stem–loop structure [72].

Formation of DNA–RNA hybrids is of importance in the replication and transcription of DNA and in the reverse transcription of viral RNA. Such hybrids can form secondary structures but they are considerably more polymorphous than DNA alone [73].

3. Properties

3.1. Physical and Chemical Properties

The size of naturally occurring DNA varies from a few thousand to 10^9 base pairs. The length of such molecules (micro- to centimeter range) can easily be measured under the electron microscope.

DNA absorbs UV light at 260–280 nm due to its bases. Aqueous DNA solutions are very viscous; viscosity depends on DNA length, DNA concentration, and temperature. Heating to a critical temperature is accompanied by a decrease in viscosity because the hydrogen bonds responsible for base pairing are disrupted and the helix structure collapses. This process is called *thermal denaturation or melting of DNA*. The temperature at which one-half of the base pairs is disrupted is denoted the *melting temperature*. It depends on the base composition (G–C pairs are more stable than A–T pairs). Double-stranded DNA ranging in size from 100 to > 100 000 base pairs melts at ca. 90 °C. In shorter double strands a gradual decrease in the melting temperature is observed. The melting temperature increases with increasing salt concentrations because the solubility of the bases decreases and hydrophobic interactions are increased. Chemicals that compete with hydrogen bond formation, such as urea or formamide, lower the melting temperature of DNA. Methanol has a similar effect; it increases the solubility of the bases and increases the interaction with water. The "melting" of double-stranded DNA is also facilitated by solvents such as ethylene glycol, dimethylformamide, dimethyl sulfoxide; low ionic strength; or extreme pH values. DNA can be denatured at an alkaline pH because the keto–enol equilibria of the bases are shifted preventing these groups from participating in hydrogen bonding.

Since the stacked bases in the double-stranded helix are not as easily excited by UV light as in single strands, absorption at 260 nm is lower for double-stranded DNA than for single strands. Increase in UV absorption can thus be used to measure DNA denaturation. At 260 nm solutions containing 50 µg/mL of double-stranded DNA, 50 µg/mL of single-stranded DNA, and 50 µg/mL of free bases have absorptions of ca. 1.00, 1.37, and 1.60, respectively.

Denaturation can also occur in the presence of proteins that destabilize the helix (melting proteins). Such proteins are required to unwind the helix during replication and to facilitate interaction between single strands during genetic recombination.

The reassociation (renaturation) of thermally denatured DNA is a spontaneous process but only occurs if the solution is cooled slowly below the melting temperature. Renaturation can take several hours, depending on the size of the molecule, because it initially relies on random base pairing (hybridization); it is, however, a cooperative process. Rapid cooling of denatured DNA at salt concentrations > 50 mmol/L produces a very compact molecule in which about two-thirds of the bases are hydrogen bonded or stacked. At salt concentrations below 10 mmol/L the DNA remains denatured even after cooling.

The length of RNA varies greatly: tRNA has a length of 75–90 nucleotides and mRNA can be up to several thousand nucleotides long. Denaturation effects are rarely observed because RNA has few truely double-stranded regions; it is most likely to be observed in tRNA.

Because they are extremely long, DNA molecules are extremely sensitive to mechanical influences (shearing forces, e.g., vigorous stirring) and easily break into small fragments (ca. 1000 base pairs). Ultrasonic treatment of DNA in solution produces fragments of ca. 100–500 base pairs owing to disruption of hydrogen bonds and single-strand and double-strand breaks in the sugar–phosphate backbone [74]. Nucleic acids are sparingly soluble in water (depending on the molecular mass). They are negatively charged and acidic at physiological pH and form water-soluble alkali and ammonium salts that can be precipitated with ethanol.

RNA and DNA are insoluble in cold acid. DNA is more sensitive to acid hydrolysis than RNA. At pH < 1, however, both DNA and RNA break down into the free bases, phosphoric acid, and (deoxy)ribose. Acid hydrolysis can be used to determine the base composition of nucleic acids (e.g., total hydrolysis can be achieved by heating DNA in 90 % formic acid at 180 °C for 30 min). The β-glycosidic linkage between the N-9 of purines and the C-1 of deoxyribose is selectively cleaved at ca. pH 4, resulting in apurinic sites. Anhydrous hydrazine cleaves the pyrimidine residues.

DNA is stable at pH 13, only 0.2 of 10^6 phosphodiester bonds are broken per minute at 37 °C. In contrast, RNA is rapidly hydrolyzed at alkaline pH.

DNA can be both specifically and nonspecifically cleaved by a variety of enzymes [deoxyribonucleases (DNases)]. RNA is cleaved by ribonucleases (RNases). Some of these cleavage reactions are exploited for sequencing RNA [75,76]. See also → Enzymes, Chap. 6.4. → Enzymes, Chap. 6.5.

3.2. Interaction with Proteins

In bacteria, DNA occurs as a complex with RNA and proteins that is bound to but not surrounded by a membrane. The DNA often has a closed circular form and is organized in a series of superhelical loops.

The DNA of higher cells is enclosed within the nuclear membrane as morphologically distinct units of varying size (chromosomes); it is associated with basic proteins called histones. The number and size of the chromosomes are species specific (karyotype). Two full turns of the DNA double helix (146 base pairs) are wound around a histone octamer (diameter ca. 8.6 nm) to form a nucleosome. The width of the grooves varies due to the periodic arrangement of A–T trinucleotides on the inside and G–C trinucleotides on the outside of the nucleosome at intervals of about ten base pairs [53]. Nucleosomes can become condensed into fibers of 10 or 30 nm (super superhelices, solenoids).

Eucaryotic cellular organelles (e.g., mitochondria, chloroplasts) possess closed circular DNA that is not associated with histones.

4. Biosynthesis and Biological Function

4.1. DNA Replication

The genetic information of all cellular organisms is stored in double-stranded DNA (viruses may, however, also have single-stranded DNA or RNA, as well as double-stranded RNA). It is extremely important that the transfer of biological information in DNA (i.e., its base sequence) occurs with a very high degree of accuracy. Because of perfected proofreading and repair mechanisms (see Section 4.5) DNA replication has an error level of 10^{-8}–10^{-11} [77], i.e., for every 10^8–10^{11} bases in newly synthesized DNA only one is incorrectly incorporated. The replication of DNA is carried out by DNA polymerases which require a single strand of DNA as a template and a short double-stranded piece of nucleic acid (formed with the help of a primer) for initiation (Fig. 6; see also → Enzymes, Chap. 6.2.).

The DNA is synthesized from deoxyribonucleotide triphosphates which are polymerized on the single-stranded DNA template with the release of pyrophosphate; the cleavage of pyrophosphate by a pyrophosphatase provides the